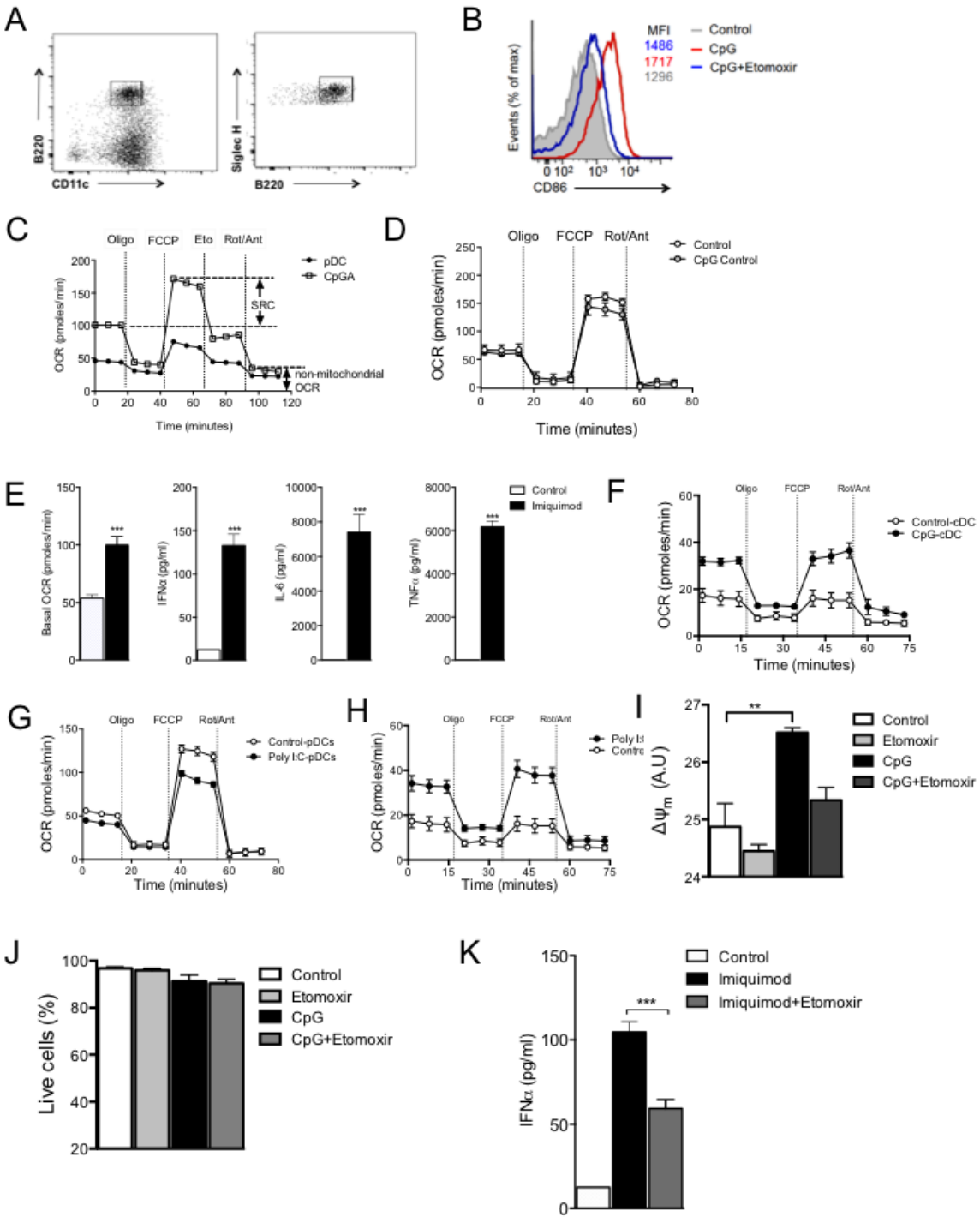
Supplem
Supplemental Information (6 figures)

Supplementary Figure S1

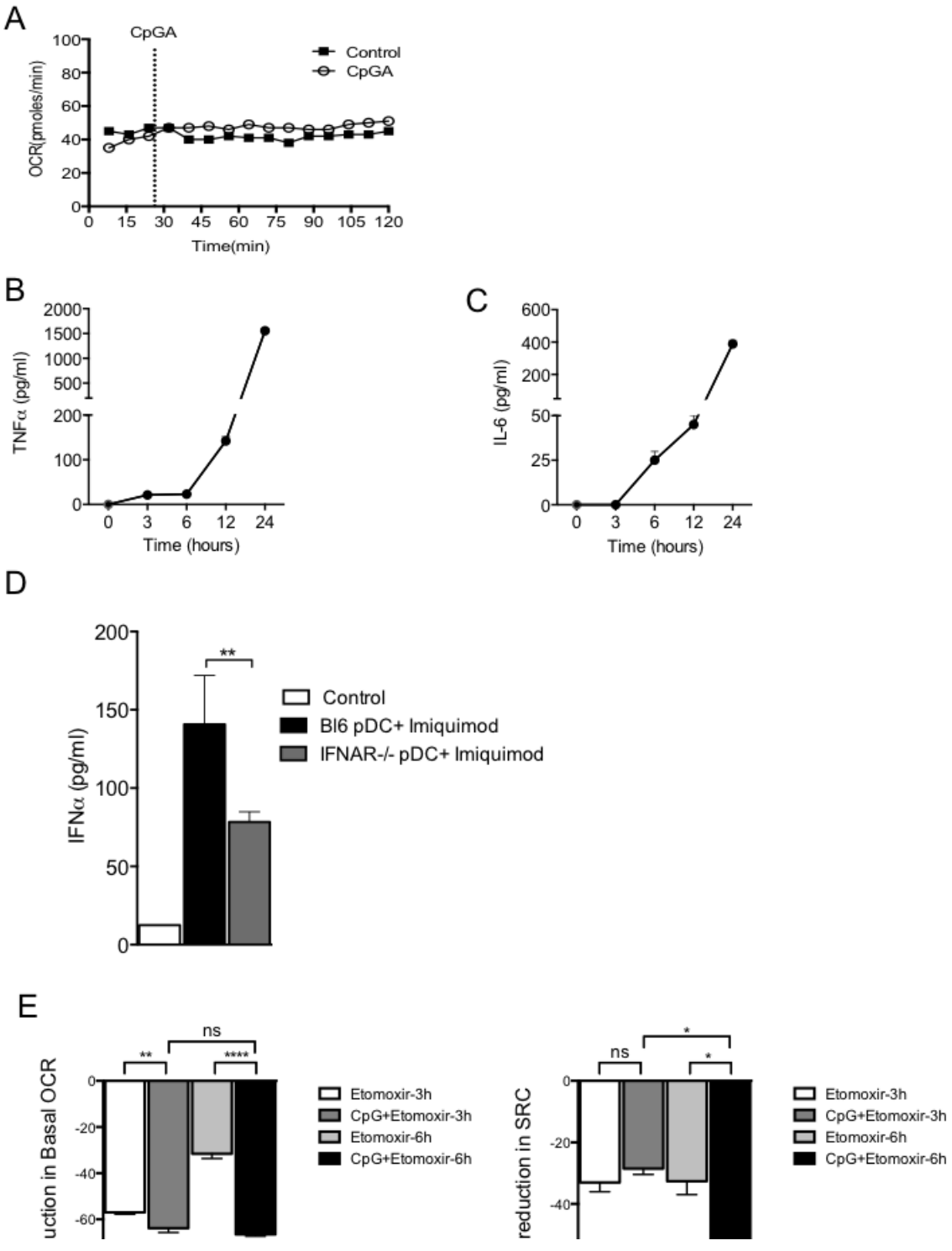


Supplemental Figure 1. Related to Figure 1.

PDC purification and activation by TLR agonists

(A) $CD11c^{int}B220^{+}$Singlec H^{+} pDCs were isolated from Flt3L-bone marrow cultures by FACS as shown. (B) pDCs were stimulated with CpGA+/- etomoxir and 24 h later analyzed for expression of CD86 by flow cytometry. MFI, mean fluorescence intensity. (C) The changes in OCR of control unstimulated pDCs and CpGA-activated pDCs were determined through a mitochondrial fitness test in which OCR is repeatedly measured at baseline and following sequential treatments with oligomycin, FCCP, etomoxir and rotenone/antimycin A. SRC, spare respiratory capacity. (D) A mitochondrial fitness test comparing OCR of unstimulated pDCs and pDCs stimulated with CpGB (CpG control). (E) Basal OCR, and IFN-α, IL-6 and TNF-α production my pDCs stimulated with imiquimod. (F) A mitochondrial fitness test comparing OCR of unstimulated cDCs and cDCs stimulated with CpGA. (G) A mitochondrial fitness test comparing OCR of unstimulated pDCs and pDCs stimulated with poly I:C. (H) A mitochondrial fitness test comparing OCR of unstimulated cDCs and cDCs stimulated with polyI:C. (I) Mitochondrial membrane potential as measured using pDCs treated as indicated. (J) Viability of pDCs treated as indicated. (K) IFN-α production by pDCs cultured without or with imiquimod or imiquimod plus etomoxir. Data points are mean ± SEM from multiple samples in one experiment, and are representative of data from 2 or more experiments Statistical significance by Student's *t*-test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Supplementary Figure S2

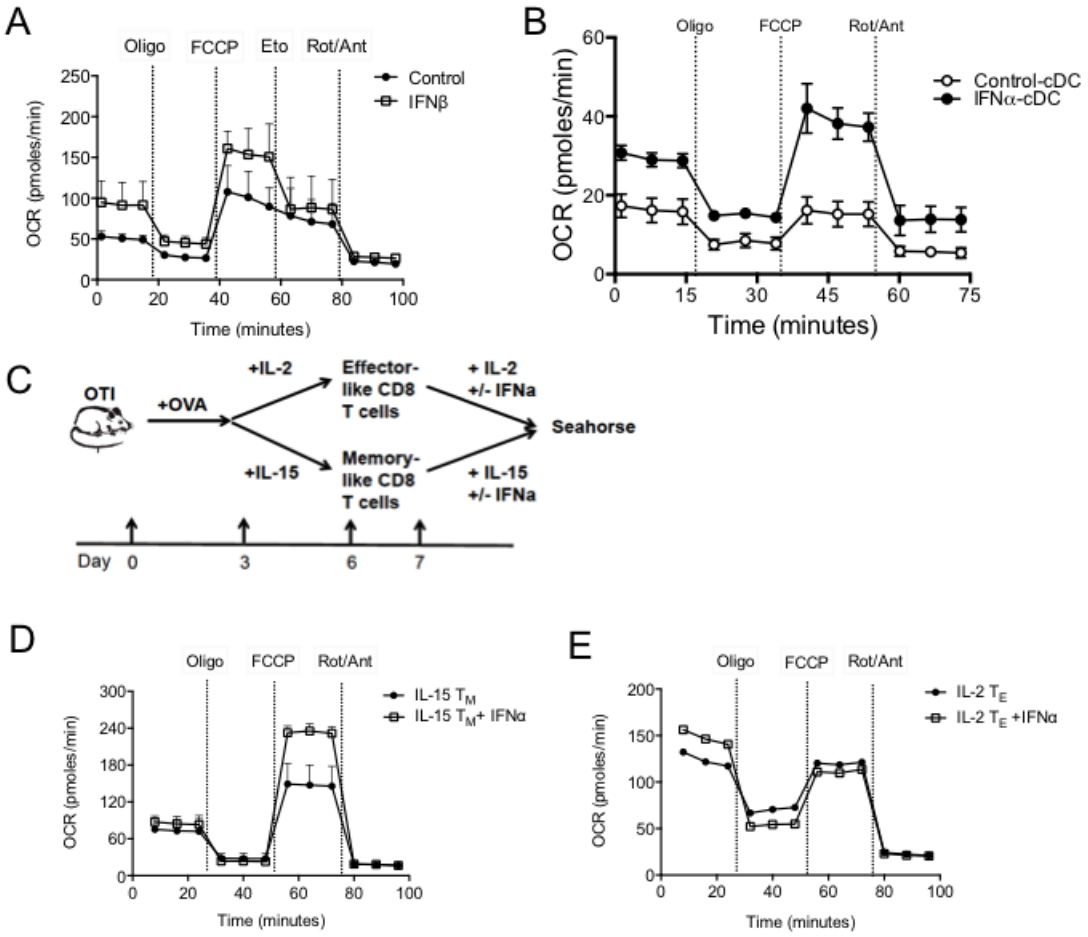


Supplemental Figure 2. Related to Figure 2

Changes in OCR and production of cytokines following stimulation with TLR agonists
(A) Real-time changes in OCR of pDCs following stimulation with CpGA. (B,C) TNF-α and IL-6 accumulation in pDC culture supernatant at 3, 6, 12, and 24 h post activation with CpGA. (D) IFN-α production by WT pDCs cultured without or with imiquimod, or by *Ifnar*-/- pDCs cultured with imiquimod. (E). Reductions in basal OCR or SRC caused by etomoxir in resting or CpGA-stimulated

pDCs at 3h and 6h post activation. Data points are mean ± SEM from multiple samples in one experiment, and are representative of data from 2 or more experiments. Statistical significance by Student's *t*-test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Supplementary Figure S3

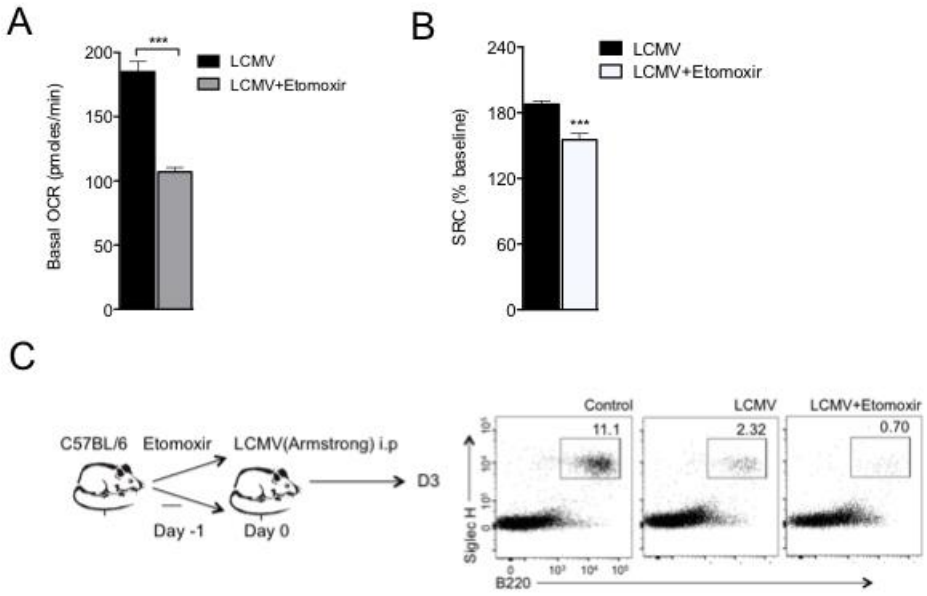


Supplemental Figure 3. Related to Figure 3

Type 1 IFNs promote FAO and OXPHOS

(A) OCR values during a mitochondrial fitness test of resting pDCs or pDCs stimulated with IFN-β for 24 h. (B) OCR values during a mitochondrial fitness test of resting cDCs or cDCs stimulated with IFN-α for 24 h (C) OT-I CD8 T cells were activated with OVA peptide and IL-2 for 3 days and subsequently cultured in IL-15 or IL-2 for 4 more days to generate IL-15 T memory (T_M) and IL-2 T effector (T_E) cells respectively. Cells were then stimulated for a further 24 h with or without the addition of IFN-α as indicated, before extracellular flux analysis. (D,E) OCR values during a mitochondrial fitness test of memory (D) or effector cells (E) cultured with or without IFN-α. Data represent mean ± SEM of reads from 5-10 samples from one experiment (D,E) or one experiment representative of 2 (A,B).

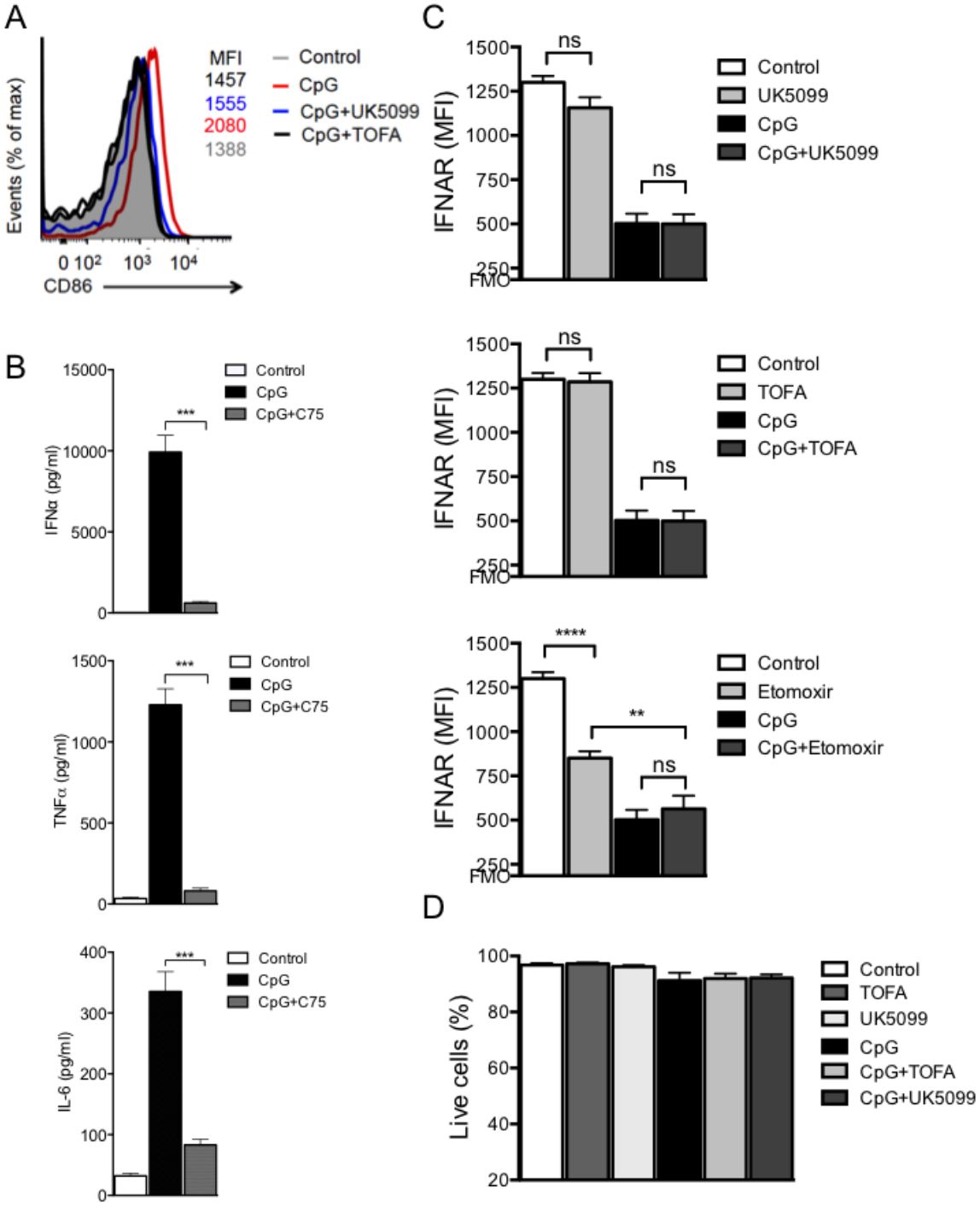
Supplementary Figure S4



Supplemental Figure 4. Related to Figure 4

In epithelial cells the production of type 1 IFNs in response to viral infection, and type 1 IFN-induced metabolic changes and inhibtion of viral replication, are inter-dependent
(A) Basal OCR of PDV cells after 24 h LCMV Arm infection in the presence or absence of etomoxir. (B) SRC of PDV cells after 24 h LCMV Arm infection in the presence or absence of etomoxir. (C) Mice were infected with 2×10^5 PFU of LCMV Arm and treated with etomoxir (20mg/kg) 1 day prior to infection and afterwards, and splenic pDCs were assessed by flow cytometry at day 3 post infection, as indicated. In A, B data are mean ± SEM of reads from 3 independent experiments. All differences shown in A-C are statistically significant (at least $p < 0.05$ by Student's t-test); *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. In (C), data are concatenated from 3 - 5 individual mice from one experiment representative of 2 experiments.

Supplementary Figure S5

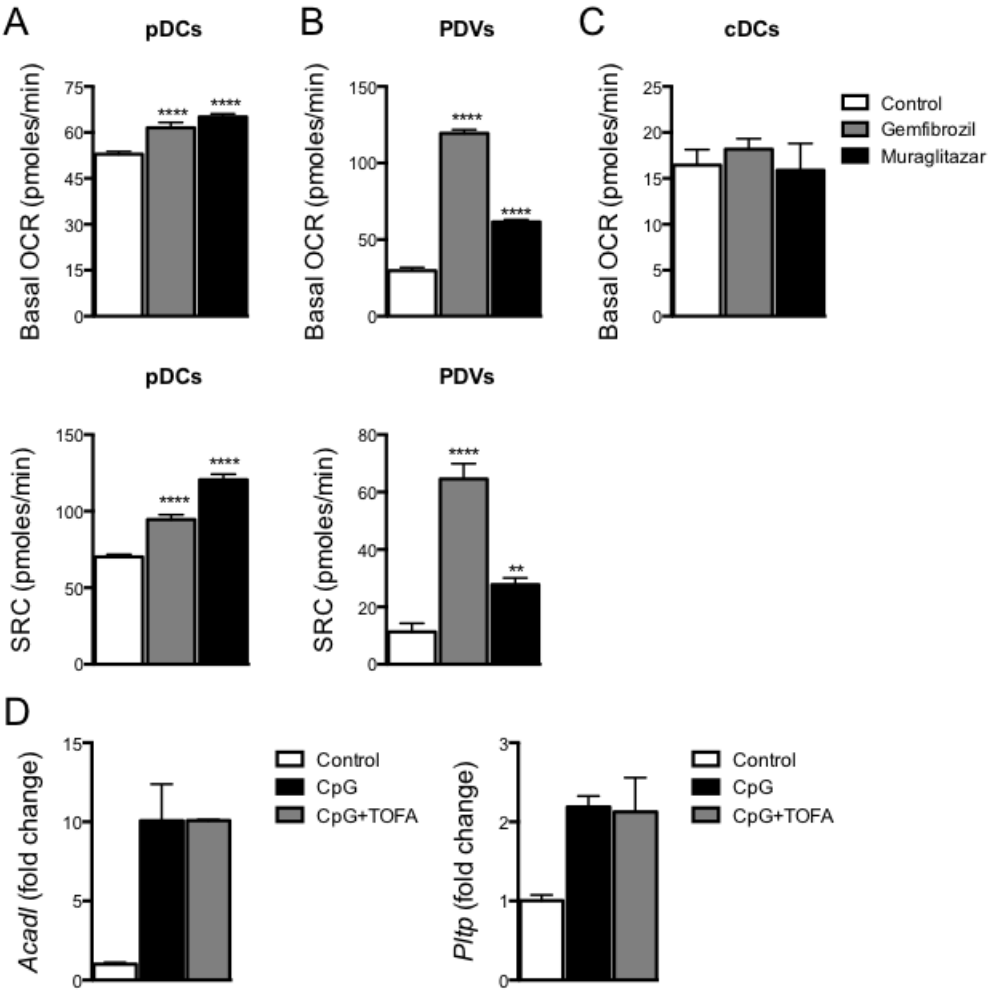


Supplemental Figure 5. Related to Figure 5

Mitochondrial pyruvate import, fatty acid synthesis and fatty acid oxidation in pDC activation
pDCs were stimulated with CpGA +/- UK5099 or TOFA or C75 and 24 h later analyzed for (A) expression of CD86 (as measured by flow cytometry); (B) production of IFN-α, TNF-α and IL-6; (C) downregulation of IFNAR (as measured by flow cytometry); (D) Viability. Data are from one individual experiment representative of two. MFI, mean fluorescence intensity. Data points are mean ±

SEM from multiple samples in one experiment, and are representative of data from 2 or more experiments. Statistical significance by Student's *t*-test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Supplementary Figure S6



Supplemental Figure 6. Related to Figure 7

PPARα agonists promote OXPHOS in pDCs and PDV cells, but not in cDCs, and on pDCs PPARα activation induced by CpGA occurs when fatty acid synthesis is inhibited.
(A) Basal OCR and SRC of pDCs in the absence or presence of Gemfibrozil or Muraglitizar. (B) Basal OCR and SRC of PDV cells in the absence or presence of Gemfibrozil or Muraglitizar. (C) Basal OCR of cDCs in the absence or presence of Gemfibrozil or Muraglitizar. (D) pDCS were cultured without stimulation, or with CpGA in the absence of presence of TOFA, and expression of PPARa target genes was measured using qRT-PCR. Data points are mean ± SEM from multiple samples in one experiment, and are representative of data from 2 or more experiments. Statistical significance by Student's *t*-test: **, $P < 0.01$; ****, $P < 0.001$.